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(54) APPARATUS FOR GEL ELECTROPHORESIS

(71) We, MILES LABORATORIES INC., a Corporation organised and existing under the laws of the State of Indiana, United States of America, of 1127 Myrtle Street, Elkhart, Indiana, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

THIS INVENTION relates to apparatus for gel electrophoresis and is more especially concerned with the provision of an apparatus for enabling the results of analytical gel electrophoresis to be translated into larger scale preparative separation. In its preferred form, as later described in detail, the apparatus is useful for studying complex proteins or enzyme systems.

It has been found that excellent resolution can be obtained of protein mixtures by electrophoresis in polyacrylamide and starch gels in analytical work but difficulties have arisen in the development of apparatus suitable for using such methods for dealing with materials in preparative quantities. Generally the technical difficulties which have arisen stem from difficulties in obtaining satisfactory elution, difficulties in mechanically stabilising a large block of gel so that it does not collapse into a collecting chamber, difficulties in dissipating heat adequately and difficulties in maintaining a uniform electrical field geometry. When referring herein to elution we are referring to the use of a flushing liquid to carry away the prepared materials.

According to the present invention there is provided apparatus for gel electrophoresis comprising a housing providing a plurality of open ended parallel chambers for the receipt of gel, a porous member providing a closure for at least one end of each chamber, an electrode chamber adjacent each of the opposite ends of the gel chambers, an elution, as hereinbefore defined, compartment located between one of the electrode chambers and an adjacent gel chamber end, the elution compartment being separated from the electrode chamber by a semi-permeable membrane and from the chamber end by the porous member, and means for feeding eluting liquid through the elution compartment. While an elution compartment can be provided common to a plurality of gel chambers separate elution compartments may be provided for individual gel receiving chambers should it be desirable to test different materials under identical conditions at the same time in the separate chambers.

Preferably a stirrer is provided in the elution compartment in order to provide consistent conditions therein and a convenient form for a stirrer comprises a paddle and means for oscillating the paddle back and forth in the elution compartment. Suitably the elution compartment may comprise an acrylic sheet of "Perspex" (Trade Mark) having an aperture therethrough, one side of the aperture being closed by a porous sheet comprising the porous support and the other being closed by the semi-permeable membrane sandwiched between two perforated support plates.

Preferably elution compartments are provided at both ends of the gel chambers whereby anodic and cathodic conditions can be examined at the same time.

Suitably the means for cooling the gel chambers may comprise passages for cooling liquid in walls separating adjacent gel chambers. Preferably means are also provided for circulating cooled buffer liquid through the electrode chambers and the cooling passages.

Means such as a peristaltic pump may be provided for feeding the eluting buffer liquid through each elution compartment in a controlled manner to a fraction collector, an

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ultra-violet photometer being provided for recording the different components removed in the elution liquid

The invention will be further described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 is an isometric view illustrating the general arrangement of electrophoretic apparatus embodying the present invention:

Figure 2 is a detailed sectional view of the semi-permeable membrane arrangement employed in the apparatus of Figure 1:

Figure 3 is a fragmentary view showing perforations in the support plate for the semi-permeable membrane:

Figure 4 is an exploded diagrammatic view illustrating the flow pattern of cooling liquid:

Figure 5 is a diagrammatic view showing the flow pattern of eluting or flushing buffer liquid;

Figure 6 is an elevational view of the sheet of perspex used to form the elution chamber; and

Figure 7 diagrammatically illustrates a stirrer for use with the elution chamber.

Referring to the drawings there is shown in Figure 1 apparatus comprising an anode chamber 2 and a cathode chamber 3 of generally similar construction. Between the chambers 2 and 3 are provided a plurality of open ended parallel chambers 4 for receipt of gel, the ends of the gel chambers being separated by an elution chamber 24 and a semi-permeable membrane assembly 6 from the electrode chamber. The electrode chambers are constructed of acrylic sheet material with the elution chamber 24 being formed by a cut-out portion of the wall 8 of the electrode chamber adjacent the gel chambers. The semi-porous membrane assembly 6 is clamped against the wall 8 by means of a compression plate 9 held at its lower edge by a block 10 secured to the inside of the bottom of the electrode chamber and at the top by an inverted U-shaped retaining block 12 clamping the wall 8 and plate 9 together. While one block 12 is illustrated in Figure 1 it will be appreciated that two such retaining blocks will be provided for each electrode chamber.

An anode 14 and a cathode 16 are illustrated extending into the anode and cathode chambers 2 and 3 respectively.

The gel chambers 4 are separated by vertical cooling walls 18 secured to a bottom cooling block 20. An upper cooling block 22 also is provided for resting on the top ends of the walls 18 whereby the top, bottome and side wall of each parallel gel chamber is constituted by a cooling wall or block.

The ends of the gel chambers 4 are closed by a porous polyethylene sheet member 26 as shown in Figures 1 and 2 for supporting gel in the chambers 2. On the side of the elution chamber 24 remote from the sheet 26 is provided the semi-permeable membrane assembly 6 which is formed by a semi-permeable membrane 27 sandwiched between two membrane mounting plates 29 sealed to the compression plate 9 by means of a gasket 28. Conical holes 30 extend through the mounting plates 29 to provide for the contact of liquid with either side of the dialysis membrane 27. The arrangement of holes 30 in the mounting plates 29 is illustrated in Figure 3.

The shape of the electrode chamber wall 8 is best seen from Figure 6 where the elution chamber is shown as being formed by a cut out section from the plate 8. Holes 32 and 34 are shown extending through the walls of the chamber 24 formed by the plate 8 and are respectively provided with nipples 36 and 38. The bottom of the chamber 24 is of wide V-shaped form sloping towards the passage 34 as shown at 39 in Figure 6.

The top of the apparatus illustrated in Figure 1 is adapted to be closed by a lid 40 of acrylic material indicated in Figure 7 which is provided with slots 41 over each of the elution compartments. A stirrer in the form of a paddle 42 extends down through the slots 41 for receipt within respective elution compartments and is carried by a rod 44 which is itself carried for oscillating movement by acrylic brackets, not shown, carried by the cover 40. Means for oscillating the stirrer paddle 42 comprises an arm 46 secured to the rod 44 displaceable by an eccentric 48 rotatable by a shaft 49. A motor, not shown, is provided for rotating the shaft 49 to cause oscillation of the stirrer paddle 42 during use of the apparatus.

Figure 4 illustrates the pattern for flow of cooling liquid through the walls of the gel chambers 4. In this Figure the top cooling block 22 is shown as being divided by baffles 50 so that liquid passed through the block will follow a serpentine path from end to end thereof. Similarly baffles 52 are provided in each of the vertical walls 18 and also baffles, not shown, are provided in the bottom cooling block 20 to ensure a serpentine flow of cooling liquid through the vertical walls 18 and the bottom cooling block 20. A pump 54 is provided for pumping the cooling liquid to a Y-piece 56 where the flow is divided, one section of the flow passing to the top cooling block 22 and from thence to the anode chamber 2 and the other portion of the liquid flow passing to the bottom cooling block 20 and vertical cooling walls 18 from whence it passes to the cathode chamber 3. From the cathode chamber the liquid flow passes to a temperature control core 58 from where it returns to the pump 54. In this Figure also suitable tubes 60 and 62 are shown for connection by a flexible tube for equalization of the liquid

levels in the two electrode chambers 2 and 3.

Figure 5 shows a flow diagram for eluting buffer liquid in which eluting buffer liquid from a reservoir 64 is caused to flow by means of a metering peristaltic pump 65 through conduits 66 and 68 to elution chambers 24. The eluting buffer liquid is then passed through conduits 67 and 69 through the proportioning peristaltic pump 65 to ultra-violet photometers from whence the flow passes to a fraction collector 72. Recorders 71 operated by the ultra-violet photometer 70 separately record the anodic and cathodic components in the flow passing to the fraction collector.

Generally flexible tubes are used for conducting eluting and cooling liquid between the various components, the components themselves being fitted with nipples, for receiving the tubing.

As indicated above a thin plate or sheet of porous polyethylene is secured to close the ends of the gel chambers. Suitably the vertical and bottom tubing walls of the gel chambers can be formed of acrylic material with the porous polyethylene plate being cemented with cyano-acrylate to these walls.

While one form of the perspex plate defining the elution chamber 4 is illustrated in Figure 6 in an alternative form instead of having the inlet for eluting buffer liquid 32 through the plate the inlet may be provided from above through a removable lid of the apparatus, this lid also mounting the stirrers.

In order to use the apparatus gel solution is supplied to the gel chambers and a former having downwardly depending members is provided as a lid so that following setting or polymerisation of the gel the former can be removed to leave slots at a suitable location in the individual gel chambers for receipt of the material to be tested. During supply of gel to the gel chambers the gel is drawn into the pores of the porous polyethylene during casting and thus gel is anchored to the porous polyethylene sheet or plate. A suitable gel separation medium is polyacrylamide as gels of this material are transparent and chemically inert; they can be varied over a wide range of concentration and pore sizes thus taking advantage of any differences in molecular size or shape as well as charge differences; furthermore polyacrylamide gels can be polymerised in the presence of many solubilising agents as urea or non-ionic detergents and over a wide range of pH, thus permitting solutions and separation of even structural proteins which are as difficult to dissolve as those of viruses, ribosomes and mitochondria and isoenzymes. When the gel solution is added to the chambers care is taken to avoid trapping air bubbles within the gel solution. For varying the dimensions of the slots in the gel as required different slot forms can be used as each slot former is relatively cheap to produce, comprising a piece of acrylic sheet having cemented thereto four downwardly depending pieces of acrylic material for receipt in the individual gel chambers. Once the slot has been formed samples can be supplied to the slot and conveniently may be supplied in agarose gel to prevent electrodecantation.

The electrical potential for electrophoresis is applied across the cathode and anode and during electrophoresis charged molecules migrate from the sample slot through the separative medium, preferably polyacrylamide gel as indicated above, to the buffer filled elution compartments. Simultaneously buffer is pumped sequentially through the elution compartments to the monitoring ultra-violet photometers and the fraction collector. During use of the apparatus the buffer in the eluting chambers is stirred by the stirring panels to prevent electrodecantation which could lead to distortion of the electrical field and thus affect the resolution both in the gel and the eluate and also to prevent absorption of proteins or other materials into the membrane. Because double ended elution is provided, both anionic and cationic species can be isolated at one time. While the construction described has been used for treating amounts of protein up to 4 grams in an alternative arrangement, by designing the plate 8 so as to provide an elution chamber or compartment for each gel chamber and by supplying separate streams of eluting buffer through the individual gel chambers, tests can be carried out simultaneously on different materials in each of the gel chambers under identical conditions for obtaining comparative results.

Thus when providing for separate collections of eluting buffer for the individual gel chambers it is possible to study four different protein/enzymes in isolation under identical conditions during the course of a single experiment.

While four gel chambers have been illustrated it will be appreciated that the capacity of the apparatus can be increased simply by building in additional gel chambers, all based on the same principle of design as outlined above with proper circulatory provision for cooling liquid.

As an example of use of the apparatus a two gram mixture of bovine plasma albumin and haemoglobin-A were fractionated. The buffer used was 0.09 M Tris, 0.002 M EDTA Na_2 and 0.089 M H_3BO_3 having a pH of 8.3. Each of the four gels comprised a 5.5% concentration of polyacrylamide, were 7.2 cm long, 2.4 cm wide and 8.0 cm deep. The sample slot was 1.8 by 0.3 cm and 7.6 cm deep: it was cast at 3.5 cm from the anodic

end of the gel bed, the width in the electrophoresis axis being 0.3 cm. The flow rate of eluting buffer was 50 ml/hour and the voltage gradient was 7 volts/cm., the temperature during electrophoresis being maintained at 4°C. Prior to the addition of the sample to the gel all the gels were pre-run for one and a half hours at 7 volts/cm., the samples being applied in 0.5 agarose (final concentration). The temperature was kept constant by the use of the temperature control coil 58 which comprised a glass coil through which the cooling buffer was flowed, the coil being immersed in a temperature control bath.

WHAT WE CLAIM IS:—

1. Apparatus for gel electrophoresis comprising a housing providing a plurality of open ended parallel chambers for the receipt of gel, a porous member providing a closure for at least one end of each chamber, an electrode chamber adjacent each of the opposite ends of the gel chambers, an elution, as hereinbefore defined, compartment located between one of the electrode chambers and and adjacent gel chamber end, the elution compartment being separated from the electrode chamber by a semi-permeable membrane and from the chamber end by the porous member, and means for feeding eluting liquid through the elution compartment.

2. Apparatus according to claim 1, wherein an elution compartment is provided common to a plurality of gel chambers.

3. Apparatus according to either preceding claim, wherein a stirrer is provided in the elution compartment.

4. Apparatus according to claim 3, wherein the stirrer comprises a paddle and means for oscillating the paddle back and forth in the elution compartment.

5. Apparatus according to any preceding claim, wherein the elution compartment comprises a sheet of acrylic material having an aperture therethrough, one side of the aperture being closed by a porous sheet comprising the porous member and the other being closed by the semi-permeable membrane sandwiched between two perforated support plates.

6. Apparatus according to any preceding claim, wherein elution compartments are provided at both ends of the gel chambers.

7. Apparatus according to any preceding claim, wherein separate elution compartments are provided for individual ones of the parallel gel receiving chambers.

8. Apparatus according to any preceding claim, wherein the means for cooling the gel chambers comprises passages for cooling liquid in walls separating adjacent gel chambers.

9. Apparatus according to any preceding claim, wherein the means for cooling the gel chambers comprises passages for cooling liquid in outer housing walls to the exterior of the gel chambers.

10. Apparatus according to claim 8 or 9, wherein means are provided for circulating cooled buffer liquid through the electrode chambers and the cooling passages.

11. Apparatus according to any preceding claim, wherein means are provided for individually feeding eluting buffer liquid in a controlled manner through each elution compartment to a fraction collector.

12. Apparatus according to claim 11, wherein a peristaltic pump is provided for feeding the eluting buffer liquid, such peristaltic pump controlling both the feeding means to and from the elution compartment.

13. Apparatus according to claim 11 or 12, wherein an ultra-violet photometer is provided for recording the different components removed in the eluting liquid.

14. Apparatus according to any preceding claim, wherein the housing and chamber components are formed of acrylic sheet material.

15. Apparatus according to any preceding claim, including gel in the gel chambers.

16. Apparatus according to claim 15, wherein the gel is provided with a slot partway along the length of each chamber for the receipt of material to be resolved.

17. Apparatus for gel electrophoresis constructed and arranged to operate substantially as hereinbefore described with reference to and as illustrated in the accompanying drawings.

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4 SHEETS

COMPLETE SPECIFICATION

This drawing is a reproduction of the Original on a reduced scale.
SHEET 1

FIG.1.

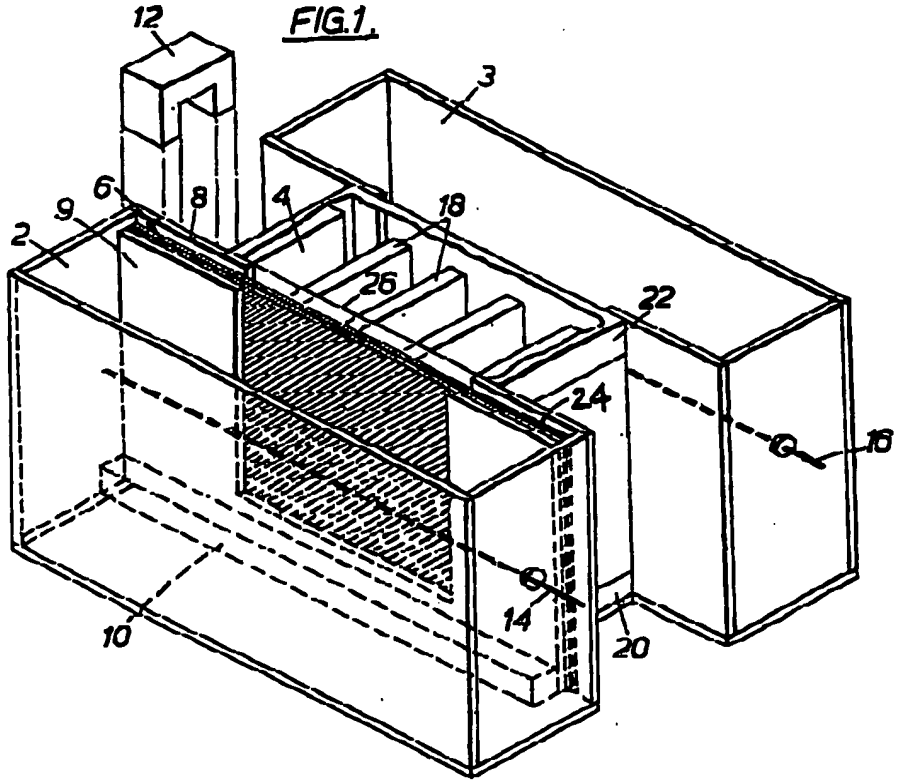


FIG.2.

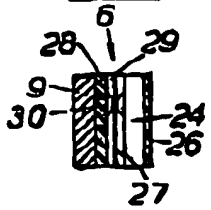
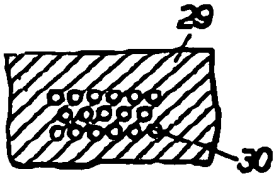


FIG.3.



1,359,944 COMPLETE SPECIFICATION
4 SHEETS This drawing is a reproduction of the Original on a reduced scale.
SHEET 2

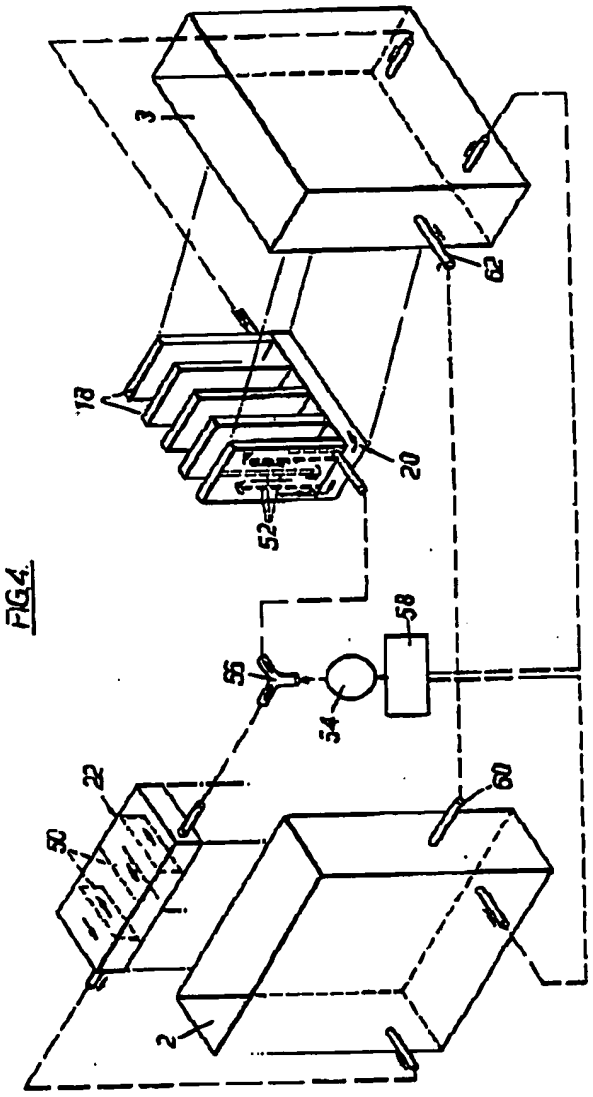


FIG.4.

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COMPLETE SPECIFICATION

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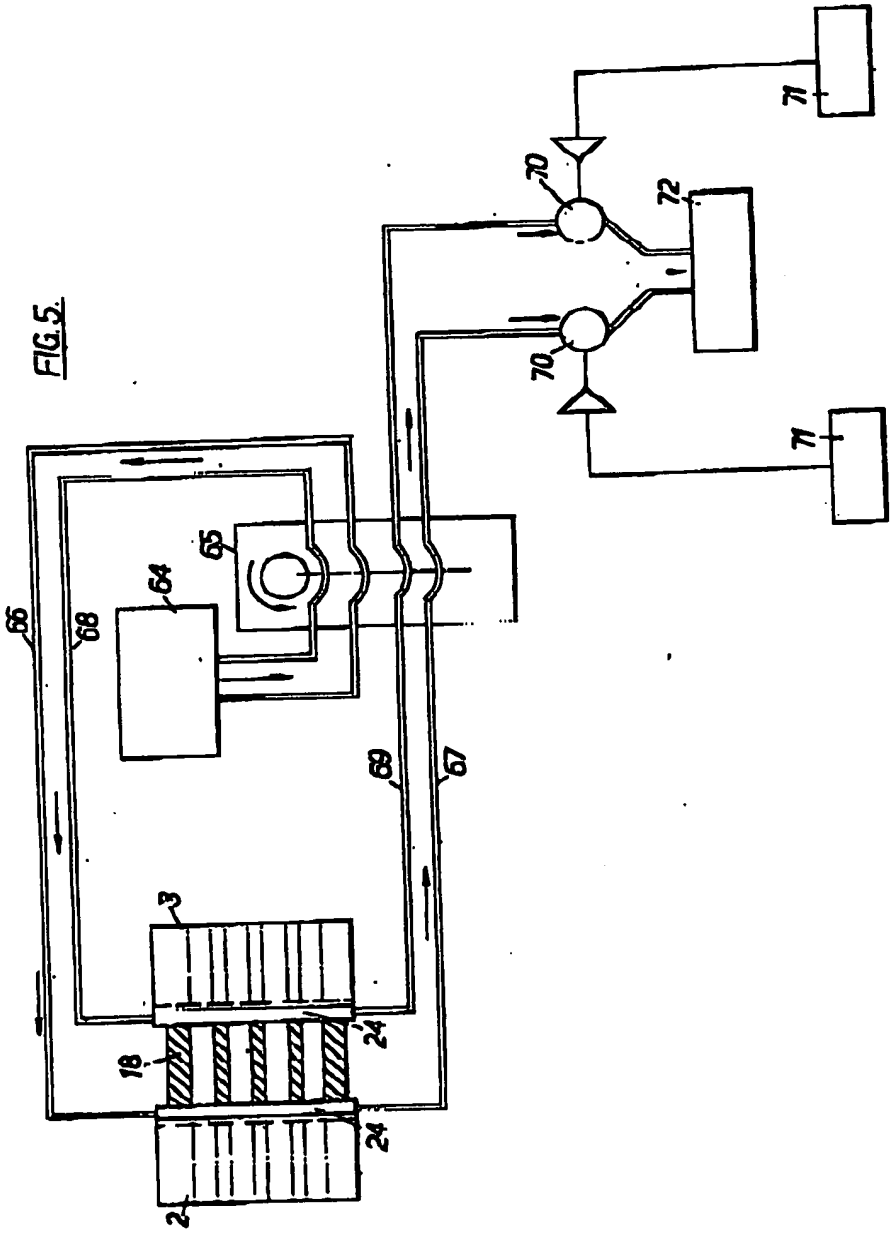
SHEET 3

FIG.5.



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SHEET 4

FIG.6.

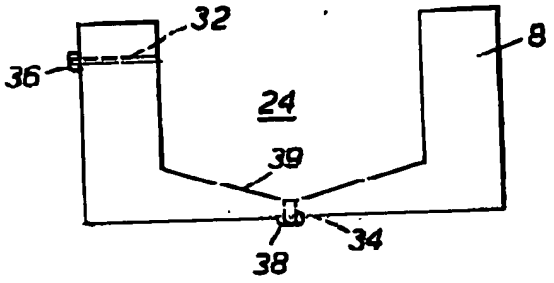

FIG.7.